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Methicillin-resistant *Staphylococcus aureus* strains in Buenos Aires Teaching Hospitals: replacement of the multidrug resistant South American clone by another susceptible to rifampin, minocycline and trimethoprim-sulfamethoxazole

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SUMMARY

The aim of this study was to characterize methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from different infectious sites of hospitalized patients at two university hospitals. Fourteen isolates were analyzed by repetitive sequence based PCR (Rep-PCR), randomly amplified polymorphic DNA assay (RAPD-PCR), and pulsed-field gel electrophoresis (PFGE). We found that a prevalent clone of MRSA, susceptible to rifampin, minocycline, and trimethoprim/sulfamethoxazole (RIF^S, MIN^S, TMS^S) was present in both hospitals in replacement of the multiresistant MRSA South American clone, previously described in these hospitals. The staphylococcal chromosomal cassette (SCC*mec*) type I element was detected in this new clone.

Key words: MRSA, mec*A*, SCC*mec*, epidemiology, molecular typing, PFGE

RESUMEN

***Staphylococcus aureus* meticilina-resistente en hospitales universitarios de Buenos Aires: reemplazo del clon Sudamericano multi-resistente por otro sensible a rifampicina, minociclina y trimetoprima-sulfametoxazol.** El objetivo de este trabajo fue la caracterización de aislamientos de *Staphylococcus aureus* meticilina-resistentes (SAMR), provenientes de diferentes procesos infecciosos de pacientes internados en dos hospitales universitarios. Catorce aislamientos fueron analizados mediante la PCR de secuencias repetitivas (Rep-PCR), la amplificación al azar de ADN polimórfico (RAPD-PCR) y la electroforesis de campo pulsado (PFGE). Encontramos que un clon prevalente de SAMR, sensible a rifampicina, minociclina y trimetoprima-sulfametoxazol (RIF^S, MIN^S, TMS^S) estaba presente en ambos hospitales, reemplazando al clon SAMR y multi-resistente previamente descrito en estos mismos hospitales. En este nuevo clon se detectó el *cassette* cromosómico estafilocócico SCC*mec* tipo I.

Palabras clave: SAMR, SCC*mec*, epidemiología, tipificación molecular, PFGE

INTRODUCTION

Staphylococcus aureus is one of the most significant pathogens causing nosocomial infections. The prevalence of methicillin-resistant *S. aureus* (MRSA) differs among different countries and different hospitals, but once MRSA strains are introduced into a hospital they may become endemic. However, MRSA are also emerging in the community and the prevalence of these strains seems likely to increase substantially (4, 17). In 2003, according to the Antibiotic Resistance Informatic System (SIR), the overall prevalence of MRSA in Argentina was estimated as 56% (Subcomisión de Antimicrobianos, Asociación Argentina de Microbiología). Hospital-associated MRSA isolates were also typically resistant to multiple, non β-lactam antibiotics. In contrast, community-acquired MRSA strains are commonly susceptible to the majority of other non β-lactams (1, 15). The extensive geographic spread of specific clones of MRSA has previously been reported, and numerous procedures have been utilized for epidemiological identification and comparison of *S. aureus* isolates. The complete characterization of MRSA clones also requires the identification of the structural types of the large *mec* element, which carries methicillin resistance determinant mec*A*. Recently, Oliveira y de Lencastre developed and validated the application of a multiplex PCR strategy for a quick characterization of the *mec* element types based on their different structural features (16).

Previous studies indicated that the multidrug resistant Brazilian clone, renamed as South American clone (only susceptible to glycopeptides), was the most disseminated in Buenos Aires (2, 6, 7). This clone contained the staphylococcal chromosomal cassette (SCC*mec*) type IIIA element (16).

The aim of this study was the molecular genotyping of MRSA isolates recovered from different infectious sites of hospitalized patients at two university hospitals, using both, unsophisticated techniques as RAPD-PCR and Rep-PCR, as well as pulsed-field gel electrophoresis (PFGE), the reference highly discriminatory method for typing MRSA isolates.

MATERIALS AND METHODS

Bacterial isolates

Twenty seven isolates of *S. aureus* recovered from hospitalized patients between April 2003 and June 2003 from two Buenos Aires teaching hospitals were included in this study. Fifteen isolates were from the Hospital de Clínicas José de San Martín (HC), a 500 bed general teaching hospital, and twelve from the Instituto de Investigaciones Médicas Alfredo Lanari (L), a 100 bed teaching hospital specialized in nephrology and renal transplantation. These are the two major hospitals of the University of Buenos Aires.

All isolates were identified by standard biochemical methods and associated with nosocomial infection according to the Center for Disease Control and Prevention definitions (11).

Six MRSA isolates (A1, A2, A3, A4, A5, and A6), previously characterized as different subclones of the South American clone were included in genotyping experiments (7).

Antibiotic susceptibility testing

Disk diffusion tests were performed as recommended by the Clinical and Laboratory Standards Institute (CLSI), ex National Committee for Clinical Laboratory Standards (NCCLS) (14). The following antibiotics were tested: oxacillin (OXA), 1 µg; ampicillin-sulbactam (AMS), 10/10 µg; erythromycin (ERY), 15 µg; gentamicin (GEN), 10 µg; minocycline (MIN), 30 µg; rifampin (RIF), 5 µg; trimethoprim-sulfamethoxazole (TMS), 1.25/23.75 µg; vancomycin (VAN), 30 µg; teicoplanin (TEI), 30 µg; ciprofloxacin (CIP), 5 µg; clindamycin (CLI), 2 µg.

Phenotypic evaluation of oxacillin resistance was performed by the oxacillin agar screen test (10, 20).

PCR for mec*A* detection and SCC*mec* assignment

Strategies for detecting mec*A* gene were performed as described elsewhere (13). *S. aureus* ATCC 25912 and *S. aureus* ATCC 43300 were used as control strains; multiplex PCR for the analysis of *mec* complex structure was performed as described by Olivieira and de Lencastre (7).

DNA purification

Bacteria were cultured overnight at 37 °C in 4 ml of Luria-Bertani broth, collected by centrifugation, resuspended in 0.1 ml of TES buffer (10 mM TrisClH pH 8.0, 1 mM EDTA, 50% sucrose) and treated with lysozyme (2 g/l) and lysostaphin (0.3 g/l) for 1 hour at 37 °C. Protoplasts were lysed by the addition of 40 µl lysis solution (10 mM TrisClH pH 8.0, 10% SDS) and 20 µl 0.5 M EDTA pH 8.0. After RNAse treatment (11 µg/ml), proteinase K was added (0.22 g/l) and extracts were incubated 60 minutes at 37 °C. DNA was purified by phenol-chloroform extractions, precipitated with ethanol and resuspended in 200 µl of MilliQ water.

PCR typing methods

Five different primers were included in the typing assays. Primers 1, 7, ERIC-2 (21) and P2 (9) were used in RAPD-PCR and RW3A (8, 22) in Rep-PCR typing.

Amplifications were performed in 50 µl of reaction buffer containing 200 µM each deoxynucleotide triphosphate, 2.5 mM $MgCl_2$, 0.5 µM primer (primer 7, 1 or ERIC 2) 0.5 U *Taq* polymerase (Invitrogen) and 1 µl DNA template. PCR mixture for RAPD-PCR with primer P2 was modified in primer concentration and units of *Taq* polymerase (1 µM and 1.25 U, respectively). Mixture for Rep-PCR contained 200 µM each deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, 1.5 µM primer RW3A, 1 U *Taq* polymerase and 1 µl DNA template.

Amplification conditions for all reactions included a first cycle of 5 min at 94 °C, 15 min at the corresponding annealing temperature, 2 min at 72°C followed by 35 cycles of 1 min at 94 °C, 1 min at the annealing temperature, 2 min at 72 °C and a final step of 5 min at 72 °C. *Taq* polymerase was added after 5 minutes of the first annealing step in all cases. The annealing temperatures were 50 °C for primer RW3A, 42 °C for P2 and 25 °C for primers 7, 1 and ERIC 2.

PCR products were separated by 1.5% agarose gel electrophoresis in TAE 1X.

Pulsed-field gel electrophoresis (PFGE) genotyping

Eleven of the 14 MRSA isolates, ten from 12 identical isolates by PCR methods and one different (isolate H322) were studied by PFGE by means of the CHEF DR-III system (Bio-Rad, Hercules, CA, USA) using a standard protocol (5). Six isolates of the South American clone were also included.

Data analysis

The relatedness among patterns was estimated by the proportion of shared bands by applying the Jaccard coefficient. Data recording and calculations were performed with RAPDistance program, version 1.04 (3), and dendrogram were constructed on the basis of the unweighted pair group method with arithmetic means (UPGMA method) included in Molecular Evolutionary Genetics Analysis software, version 1.02 (12).

RESULTS AND DISCUSSION

From 27 isolates included, 14 were MRSA. The susceptibility pattern of these isolates is presented in Table 1 and antibiotic susceptibility of methicillin-susceptible *S. aureus* (MSSA) in Table 2. The majority of the MRSA isolates were susceptible to vancomycin, rifampin, trimethoprim-sulphamethoxazole, teicoplanin and minocycline. Oxacillin agar screen test could detect all MRSA isolates, confirmed by PCR for the mec*A* gene.

By multiplex PCR was determined the structural type of the *mec* element; 13 of 14 MRSA contained SCC*mec* I and one isolate (H322) contained SCC*mec* IV (Figure 1). MRSA isolates of the multiresistant South American clone, previously detected as prevalent in the same hospitals, contained SCC*mec* IIIA as it was described previously (16).

PCR-based typing methods were performed on MRSA and MSSA isolates. A cut-off point of 85% was considered to define types which were coded with capital letters, and different profiles within each type were considered to represent subtypes (capital letters with numerical

Table 1. PCR genotyping results and antibiotic susceptibility pattern for methicillin resistant *S. aureus* isolates.

Isolate	Origin	mecA	Typing pattern by					Susceptibility pattern
			Primer 1	Primer 7	Eric-2	P2	RW3A	
61622	HC	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI
28396	HC	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI
60265	HC	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI
600130	HC	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI
60236	HC	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI
11000	HC	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI, CIP
H172	L	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI
L104	L	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI
C155	L	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI
29646	HC	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI
50122	HC	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI
60571	HC	+	A1	A1	A1	A1	A1	MIN, RIF, TMS, VAN, TEI
72547	HC	+	J1	H	I1	K	G	MIN, TMS, VAN, TEI
H322	L	+	B	B1	C2	I	D1	MIN, RIF, TMS, CIP, CLI, VAN, TEI

Table 2. PCR genotyping results and antibiotic susceptibility pattern for methicillin-susceptible *S. aureus* isolates.

Isolate	Origin	mecA	Typing pattern by					Susceptibility pattern
			Primer 1	Primer 7	Eric-2	P2	RW3A	
61599	HC	-	J2	G	G	N	F	OXA, AMS, ERY, MIN, RIF, TMS, CIP, VAN, TEI
72525	HC	-	G	D3	I2	J	C	OXA, AMS, ERY, MIN, RIF, TMS, GEN, VAN, TEI
72534	HC	-	K	I	H	L	H	OXA, AMS, ERY, MIN, RIF, TMS, GEN, VAN, TEI
H146	L	-	J2	D2	A4	A1	A1	OXA, AMS, ERY, CLI, MIN, RIF, TMS, CIP, GEN, VAN, TEI
H229	L	-	L	D1	A4	C1	E1	OXA, AMS, ERY, CLI, MIN, TMS, GEN, CIP, VAN, TEI
H235	L	-	H1	E1	B2	A3	D3	OXA, AMS, ERY, CLI, MIN, RIF, TMS, CIP, GEN, VAN, TEI
L82	L	-	I	E2	A3	C2	E2	OXA, AMS, CLI, MIN, RIF, TMS, CIP, GEN, VAN, TEI
L83	L	-	H2	D1	B1	C2	B1	OXA, AMS, ERY, CLI, MIN, RIF, TMS, CIP, GEN, VAN, TEI
28985	HC	-	E1	F	C1	A4	A2	OXA, AMS, ERY, CLI, MIN, RIF, TMS, GEN, VAN, TEI
33000	HC	-	A2	F	-	B	A1	OXA, AMS, ERY, CLI, MIN, RIF, TMS, CIP, GEN, VAN, TEI
H311	L	-	F1	C1	A2	M	B1	OXA, AMS, ERY, CLI, RIF, GEN, VAN, TEI
L141	L	-	C	C2	A5	-	B2	OXA, AMS, ERY, CLI, MIN, RIF, TMS, CIP, VAN, TEI
L139	L	-	D	B2	A5	-	B3	OXA, AMS, ERY, CLI, MIN, RIF, TMS, CIP, VAN, TEI

HC, Hospital de Clínicas José de San Martín; L, Instituto de Investigaciones Médicas Alfredo Lanari; MIN, minocycline; RIF, rifampin; TMS, trimethoprim-sulfamethoxazole; VAN, vancomycim, TEI, teicoplanin; CIP, ciprofloxacin; CLI, clindamycin; OXA, oxacillin; AMS, ampicillin-sulbactam; ERY, erythromycin ; GEN, gentamicin.

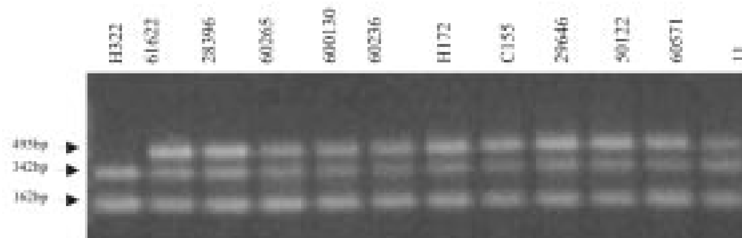

Figure 1. SCC*mec* multiplex PCR. Lane 1, SCC*mec* type IV (isolate H322); lane 2 to 12, SCC*mec* type I (10 isolates of the new clone)

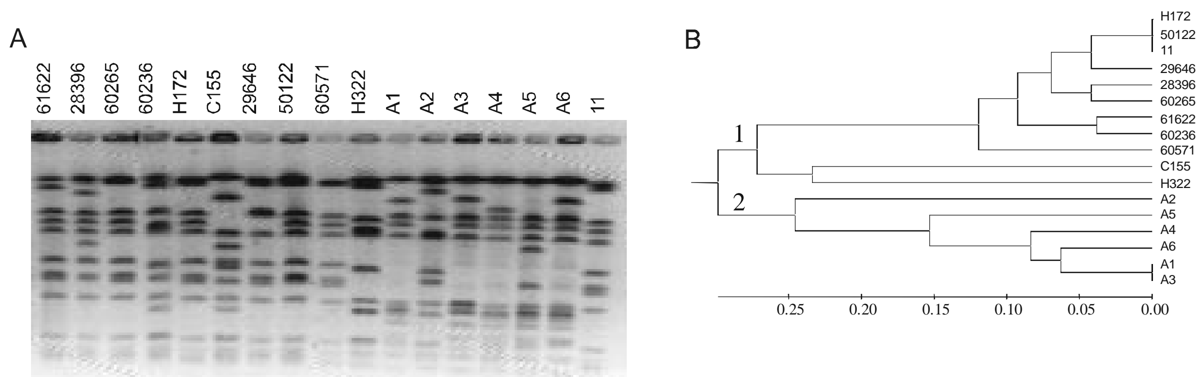

Figure 2. A. PFGE band patterns of 11 MRSA isolates recovered between April-June 2003 in two University hospitals of Buenos Aires and six isolates of the South American clone previously detected as prevalent at these hospitals (A1, A2, A3, A4, A5, and A6) B. Clonal relationships established with SmaI PFGE analysis. The scale indicates the percent of similarity according to the Jaccard coefficient within this set of strains.

subscripts). Indistinguishable patterns were obtained for 12 of 14 MRSA isolates by every PCR-based typing method, producing identical grouping (Table 1). The six MRSA isolates of the South American clone previously detected as prevalent at these hospitals (6, 7) exhibited a different type pattern by these methods (data not shown). MSSA isolates (used as a control for their expected diversity) presented a high variety of types and subtypes (Table 2). These results show the ability of these methods to differentiate among unrelated strains.

Dendrogram of PFGE-constructed on the basis of similarity of levels are shown in Figure 2. At 70% similarity two clusters of MRSA isolates were defined. Cluster 1 included all MRSA recovered between December 2002 and July 2003 meanwhile MRSA of the South American clone were grouped in cluster 2. One MRSA isolate (H322) belonging to a different type by PCR typing was also included in cluster 1 by PFGE, but its inclusion was borderline with our empirical cut-off. This isolate was also the only carrying a different *mec* cassette.

Several works have compared different PCR based typing methods with PFGE, considered the gold standard for molecular typing MRSA (19, 21). The PCR methods used in the present work were able to identify a prevalent clone and to differentiate it from the other isolates. These methods proved to be suitable to be employed in clinical epidemiological settings allowing the prompt identification of sporadic, endemic and epidemic clones. Although PFGE detects minor variants among isolates showing indistinguishable patterns by PCR-based methods, the main disadvantages are high initial cost due to the equipment required and the time-consuming procedure. Molecular typing methods based on DNA amplification by PCR are faster and less expensive

S. aureus isolates causing hospital infections within the studied period revealed the prevalence of a MRSA clone not previously described at these teaching hospitals. In a previous study, the Brazilian epidemic clone has been detected as predominant in these hospitals (6, 7), but the clone detected in this work has a different antibiotic susceptibility pattern, a different SCC*mec* element, and its different genotype was confirmed by PCR typing methods and PFGE. In 2002, a clone with an identical antibiotype was described in Córdoba province, in our country (18). Further studies are needed to explain the displacement of one clone by another in the hospital environment. Multiple factors as virulence, stability, capacity to survive in adverse environment conditions, and to colonize patients and hospital personnel, minor requirements of internal energy, considering that the new clone

is more susceptible to certain antibiotics, and finally, the selective antibiotic pressure, probably had contribute to its emergence, spreading, and predominance.

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